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Original Article

Improved repeatability of nasal potential difference with a larger surface catheter

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Abstract

Objective: To increase the power of nasal potential difference (NPD) as a biomarker of CFTR function, improvement of its repeatability is needed. We evaluated the improvement in repeatability resulting from measuring NPD (1) over a larger surface area and (2) at a fixed location.

Methods: To assess repeatability, NPD was measured on two occasions with a new method using a larger surface catheter at fixed locations on the nasal floor (LSC-floor$_{5cm}$ and LSC-floor$_{3cm}$) or at the most negative basal potential (LSC-floor$_{max}$); with a sidehole catheter on the nasal floor at 5 cm from the nasal margin (SHC-floor$_{5\ cm}$) or at the most negative potential (SHC-floor$_{max}$); and with an endhole catheter below the inferior surface of the lower turbinate at the most negative potential (EHC-turb$_{max}$).

Results: The within-subject standard deviation (S_w) for repeated measurements of the total chloride response in the controls was smallest with the LSC-floor at a fixed location (LSC-floor$_{5\ cm}$ 3.1 mV; 95% CI 2.3–4.6 mV) and highest with the SHC-floor (SHC-floor$_{max}$ 14.6 mV; 95% CI 10.9–22.2 mV) or the EHC-turbinate (EHC-turb$_{max}$ 12.5 mV; 95% CI 10.7–23.0 mV) at the most negative basal potential. Measuring with the LSC-floor at the maximal potential increased the S_w (LSC-floor$_{max}$ 8.8 mV, 95% CI 6.0–16.1 mV, p = 0.009 vs LSC-floor$_{5\ cm}$), while measuring with the SHC-floor at a fixed location slightly decreased the S_w (SHC-floor$_{5\ cm}$ 9.8 mV, 95% CI 8.9–20.6 mV, p = 0.06 vs SHC-floor$_{max}$). In patients with cystic fibrosis, the S_w was comparable, between 2.2 mV and 4.3 mV. Sample size calculations for trials using NPD to assess changes in ion transport showed that the number of subjects to be included could be approximately halved measuring with the larger surface catheter at a fixed location vs SHC or EHC at fixed locations.

Conclusion: Measuring the NPD at a fixed location and over a larger surface resulted in increased repeatability and thereby also power as a biomarker of CFTR modulation.

Keywords: Surrogate endpoint; CFTR function; Biomarker

1. Introduction

In patients with cystic fibrosis (CF), defective chloride transport through the cystic fibrosis transmembrane conductance regulator (CFTR) is the initiator of a pathophysiological cascade leading to respiratory disease. The first therapy targeting the basic CF ion transport defect was recently licensed. Ivacaftor improves chloride transport [1] and also improves lung function, increases weight and decreases the exacerbation rate in patients harboring the G551D mutation [2]. Therapies targeting the CFTR defect in patients with other CF mutations are under development. Lumacaftor aims to increase the amount of CFTR protein at the

Abbreviations: CF, cystic fibrosis (CF); CFF, the Cystic Fibrosis Foundation; CFF-TDN, Cystic Fibrosis Foundation Therapeutic Development Network; CFTR, cystic fibrosis transmembrane conductance regulator; ECFS, the European Cystic Fibrosis Society; ECFS-CTN, European Cystic Fibrosis Society Clinical Trial Network; NPD, nasal potential difference; SOP, standard operating procedure; Sw, within-subject standard deviation; TCR, total chloride response.

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cell membrane in F508del patients [3], and ataluren promotes the read-through of premature nonsense mutations [4].

These therapies are examples of personalized medicine because they are geared towards patients with a specific mutation class. While phase 3 efficacy trials with CFTR modulators are feasible for frequent *CFTR* mutations, such trials will be more challenging for rare mutations.

Therefore, biomarkers that predict the effects of these new therapies in patients with rare mutations are needed [5]. The nasal potential difference (NPD) directly measures the ion transport in the airways and is consistently abnormal in patients with CF [6]. In early studies, aminoglycosides [7] and ataluren [8] have been shown to improve chloride transport in patients with nonsense mutations. Treatment with ivacaftor partially corrects the chloride and sodium transport defects observed on NPD in G551D patients [1].

However, NPD measurements are highly variable [9–11], leading to decreased power to detect changes with treatment [12]. To improve the repeatability of the measurements, standard operating procedures (SOPs) were devised [13,14], the central reading of tracings during clinical trials was instituted [1], and the processing of the obtained values was optimized [15]. Despite these measures, the NPD measurement variability remains high.

The goal of the present study was to compare a new method of measuring the NPD with previously described measurement methods, with regard to repeatability. Two major changes were introduced in the measurement technique. First, the measurement surface area was increased to average the potentials from a larger area of the epithelium. Second, the repeat measurements were performed at a fixed location instead of at the spot of the most negative basal potential. Our hypothesis was that these technical modifications would increase the repeatability of the NPD measurement and thus the power to detect therapeutic effects.

2. Methods

2.1. Subjects

Patients with CF were recruited during outpatient clinic visits or during admission. The control subjects were recruited through an advertisement. All of the subjects had to be free of any acute upper airway symptoms for more than two weeks. Smokers were excluded. The ethics committee of the University of Leuven approved the study. Caregivers and/or the participants gave written informed consent. Subjects could contribute to the evaluation of more than one measurement method, but not all methods were evaluated in each subject.

2.2. Nasal potential difference

The NPD was measured between an intranasal agar-filled catheter and an agar-filled subcutaneous needle (21G or 23G). Potentials were recorded with calomel electrodes (Calomel Reference Electrode, Radiometer Analytical, Villeurbanne, France) connected through a head stage to a bio-amplifier (ISO-Z Isolated Head-Stage and BMA-200 AC/DC Portable Preamplifier, AD Instruments, New Zealand) and a digital recorder (Powerlab 4/30, AD Instruments, New Zealand), as described in both of the available SOPs [13,14].

The potential was measured sequentially during perfusion at 5 ml/min with Ringer's solution, Ringer's solution with 100 μM amiloride, a zero-chloride solution with 100 μM amiloride and a zero-chloride solution with 100 μM amiloride and 10 μM isoprenalin. Each solution was perfused for 3 min or until a stable potential value was obtained, whichever was longer. Solutions were not warmed, as only a minor effect is expected on the potential measurements [16].

Three main indices were calculated: (1) the total chloride response (TCR) was the sum of the change in the NPD observed after changing from the amiloride in Ringer's solution to the amiloride in zero-chloride solution plus the change after changing to the isoprenalin plus amiloride in zero-chloride solution; (2) the Ringer's PD was the NPD at the end of the perfusion with Ringer's solution; and (3) the delta NPD was the change in the NPD from the end of the perfusion with Ringer's solution to the end of the perfusion with the isoprenalin plus amiloride in zero-chloride solution.

Tracings with both the amiloride response lower than 5 mV and the TCR higher than −5 mV were considered 'flat tracings' and were not used for further analysis.

The following different methods were compared (Table 1).

1. The new 'larger surface catheter' method (LSC-floor$_{5\ cm}$): the NPD was measured with a larger surface agar-coated electrode (agar coating of 2 cm length over 360° with a diameter of 4.5 mm, Fig. 1 and Fig. S1 in the online supplement) secured with the tip 5 cm past the nasal margin.
2. SHC-floor$_{max}$: the NPD was measured along the nasal floor with an 8 Fr single lumen side-hole nasogastric catheter (Fig. 1). The solutions were perfused through polyethylene PE90 tubing ending near the opening of the measuring electrode, attached by a short silicone sheat. The catheter was secured at the site of the most negative basal potential. This measurement method is close but not identical to the ECFS-CTN SOP [13], which advocates the use of a sidehole Marquat catheter while a home-made sidehole catheter was used in the present study.
3. EHC-turb$_{max}$: the NPD was measured at the lower surface of the inferior turbinate with an end-hole 2.5-mm catheter (Marquat Genie Biomedical, Boissy-Saint-Léger, France, Fig. 1). Perfusion of the solutions was achieved through the second lumen of the catheter. The catheter was inserted under visualization with a nasal speculum and a headlamp and was secured when the location with the most negative basal potential was identified. This method is similar to the one used for the ataluren trial [8] and differs from the actual CFF-TDN SOP in two ways: the solutions were not warmed and a Marquat endhole catheter was used instead of a PE90/PE50 home-made catheter.
4. Additional methods were tested to assess the respective contributions of the two modifications — larger surface and fixed location — to the improvement in repeatability:

   ◦ SHC-floor$_{5\ cm}$: the sidehole catheter was tested with the catheter secured at a fixed location on the nasal

Table 1
Comparison between the Ringer's PD values and the total chloride response measured with different methods in control subjects and patients with cystic fibrosis.

Method			LSC-floor$_{5\ cm}$	SHC-floor$_{max}$	EHC-turb $_{max}$	SHC-floor$_{5\ cm}$	LSC-floor$_{3\ cm}$	LSC-floor$_{max}$
Catheter type			Larger surface	Side-hole	End-hole	Side-hole	Larger surface	Larger surface
Location			Nasal floor 5 cm	Nasal floor Most neg PD	Inferior turbinate Most neg PD	Nasal floor 5 cm	Nasal floor 3 cm	Nasal floor Most neg PD
No. flat tracings/total no. of tracings			2/54	7/60	1/46	6/34	14/100	0/20
Total chloride response (mV)	Controls	No. of valid tracings	36	35	27	12	67	20 **
		Mean	−22.9	−24.2	−36.6 ****	−40.1 ***	−13.3 *	-15.2
		SD	9.0	15.2	13.8	12.5	7.3	7.7
		Mean difference d2-d1	−1.2	−4.7	0.1	3.8	−1.2	−4.2
		SD difference d2-d1	4.4	20.8	18.3	15.0	6.3	12.4
		Within-subject SD	3.1	14.6 ****	12.5 ****	9.8 ****	4.5	8.8 ****
	CF	No. of valid tracings	16	18	18	16	19	
		Mean	0.8	1.4	−2.8	1.4	2.3	
		SD	5.6	3.7	4.6	8.7	2.3	
		Mean difference d2-d1	0.9	−2.6	−2.0	0.5	−1.7	
		SD difference d2-d1	5.3	3.8	6.0	6.4	2.9	
		Within-subject SD	3.3	3.1	4.3	4.2	2.2	
Ringer's PD (mV)	Controls	No. of valid tracings	36	35	27	12	67	20
		Mean	−11.9	−14.6	−22.5 ****	−10.5	−10.0	−13.3
		SD	6.1	7.2	11.2	4.8	3.8	4.3
		Mean difference d2-d1	−1.5	−1.1	0.3	0.8	0.4	−0.3
		SD difference d2-d1	4.5	4.7	4.9	6.0	2.0	5.0
		Within-subject SD	3.0	3.3	3.4	3.8	1.4	3.4
	CF	No. of valid tracings	16	18	18	16	19	
		Mean	−48.2	−40.6	−58.9	−55.1	−29.2	
		SD	16.8	13.7	11.8	12.7	9.1	
		Mean difference d2-d1	4.4	11.6	−0.9	2.6	3.2	
		SD difference d2-d1	9.3	14.2	14.8	17.1	5.8	
		Within-subject SD	6.9	12.5	9.9	11.4	4.5	

CF: cystic fibrosis; EHC: endhole catheter; LSC: larger surface catheter; neg PD: negative potential difference; SHC: sidehole catheter; turb: inferior surface of the inferior turbinate; TCR: total chloride response. Only the differences between each method and the new LSC-floor$_{5\ cm}$ method were assessed.

* $p < 0.05$.

** $p < 0.01$.

*** $p = 0.001$.

**** $p < 0.001$ compared with the new method.

floor (5 cm), similar to the procedure in the new method.

- LSC-floor$_{3\ cm}$: the new larger surface catheter was used with the tip secured 3 cm past the nasal margin.
- LSC-floor$_{max}$: the new larger surface catheter was used with the catheter secured at the site of the most negative basal potential, similarly to the SHC-floor$_{max}$ and EHC-turb$_{max}$ methods.

In each subject, both nostrils were measured simultaneously using a different technique. For the EHC-turb$_{max}$ only one nostril was tested because testing the other nostril at the same time with another technique would have compromised the precise location of the catheter.

2.3. Statistics

To estimate the repeatability, the mean difference plus the standard deviations of the difference and a Pearson's correlation between the repeated measurements were calculated. Bland and Altman plots with 95% limits of agreement were computed. In addition, a one-way ANOVA using subject as factor was used to estimate the within-subject standard deviation [17], separately for each method as not all methods were evaluated in each subject. The variances were compared using Levene's test. Fischer's exact test was used to compare the proportion of flat tracings between the methods. The mean values obtained with each method were compared using ANOVA and post-hoc Scheffé testing. Power calculations were carried out based on the means and standard deviations (SDs) of the differences between the measurements of the control subjects and the patients with CF. The power was calculated for the treatment effects predicted to correct the PDs by 10, 20 and 30% towards the values in the control subjects for a 1-sample t-test with a significance level of 0.05 and a power of 0.90.

3. Results

The nasal potential difference was measured in 113 subjects (76 controls and 37 patients with CF). The median number of methods tested in each subject was 2 (range 1–5). After a

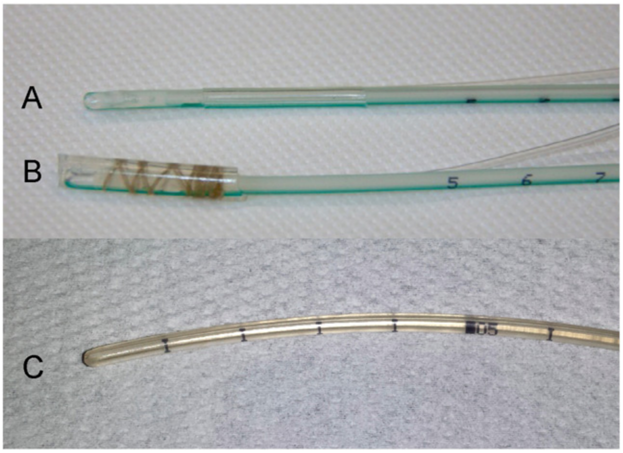

Fig. 1. Catheters used for the nasal potential difference measurements: (A) an 8 Fr side-hole catheter used for the SHC-floor method; (B) a larger surface catheter used for the new LSC-floor method; and (C) a Marquat catheter used for the EHC-turb method. EHC: endhole catheter; LSC: larger surface catheter; SHC: sidehole catheter; turb: inferior surface of the inferior turbinate.

minimum of 1 day (median 3 days), the measurements were repeated using the same method in the same nostril.

3.1. Repeatability

The means and SDs of the differences between the repeated measurements and the within-subject standard deviations are given in Table 1. There were no systematic differences between the repeated measurements of the TCR or the Ringer's PD for all of the techniques (Table 1 and Fig. 2). For the TCR in the controls, the within-subject SD was smaller when the potential was measured with the new method (LSC-floor$_{5\ cm}$ 3.1 mV, 95% CI 2.3–4.6 mV) than with the SHC-floor$_{max}$ method (14.6 mV, 95% CI 10.9–22.2 mV, $p < 0.001$) or with the EHC-turb$_{max}$ method (12.5 mV, 95% CI 10.7–23.0 mV, $p < 0.001$). Measuring with the larger surface catheter at the location of the most negative potential instead of at a fixed location resulted in a higher within-subject SD (LSC-floor$_{max}$ 8.8 mV, 95% CI 6.0–16.1 mV, $p = 0.009$), while measuring with the side-hole catheter at a fixed location instead of at the site of the most negative basal PD also reduced the within-subject standard deviation (SHC-floor$_{5\ cm}$ 9.8 mV, 95% CI 8.9–20.6 mV, $p = 0.06$).

In the patients with CF, the within-subject SDs for the TCR were similar between the different techniques (from 2.2 to 4.3 mV).

The correlation between the repeated measurements of the TCR (Fig. 3) was 0.95 (95% CI 0.91–0.98) with the new LSC-floor$_{5\ cm}$ method, 0.57 (95% CI 0.22–0.77) with the SHC-floor$_{max}$ method and 0.76 (95% CI 0.58–0.90) with the EHC-turb$_{max}$ method.

The results for the Ringer's PD and delta NPD are shown in Table 1, Table S1 and Figures S2 to S6.

3.2. Flat tracings

The number of flat tracings obtained with each method is given in Table 1. There were more flat tracings using a side-hole catheter on the nasal floor than using an end-hole catheter under the inferior turbinate (13/94 vs. 1/46, $p = 0.035$). At 5 cm from the nasal margin, the use of a larger surface catheter resulted in marginally less frequent flat tracings compared with the use of a side-hole catheter (2/54 vs. 6/34, $p = 0.05$).

3.3. Discriminative power between patients with CF and controls

All of the measurement techniques differentiated well between the control subjects and the patients with CF (Fig. 4), with some overlap for the TCR and the Ringer's PD. As a combined index reflecting the sodium and chloride transport, the delta NPD was even more discriminative (Figure S2).

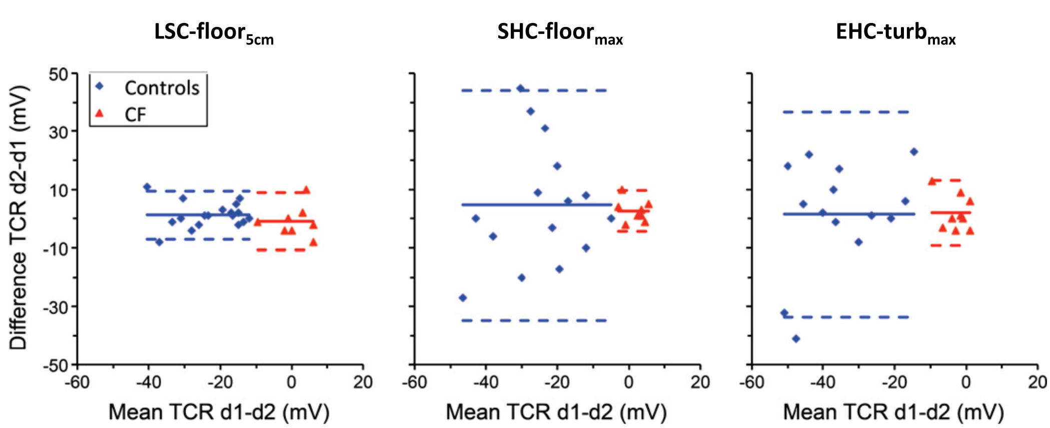

Fig. 2. Bland and Altman plots comparing the TCR measured on day 1 (d1) and day 2 (d2) in the same nostril with the same technique in the control subjects (blue diamonds) and in patients with CF (red triangles). The solid lines are the mean differences, and the dashed lines are the 95% limits of agreement for the control subjects (blue) and patients with CF (red). CF: cystic fibrosis; EHC: endhole catheter; LSC: larger surface catheter; SHC: sidehole catheter; turb: inferior surface of the inferior turbinate; TCR: total chloride response.

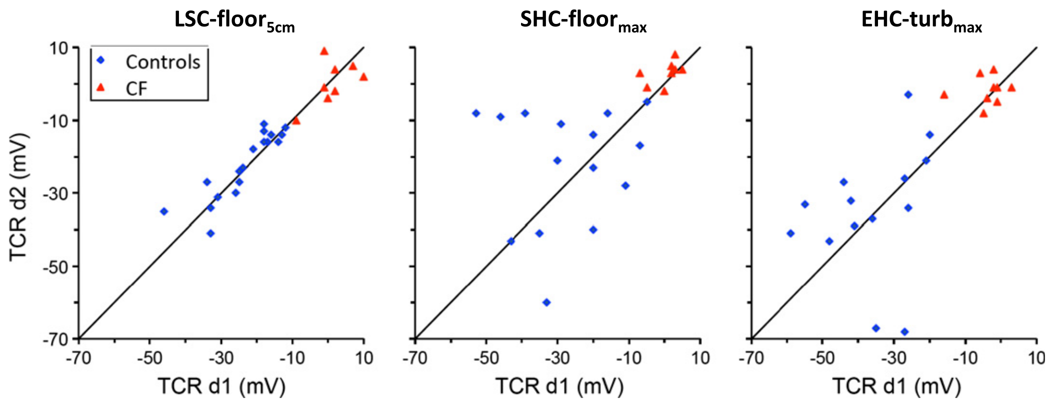

Fig. 3. Correlation between the TCR measured on day 1 (d1) and day 2 (d2) in the same nostril with the same technique in the control subjects (blue diamonds) and CF patients (red triangles). The solid line is the line of equality. CF: cystic fibrosis; EHC: endhole catheter; LSC: larger surface catheter; SHC: sidehole catheter; turb: inferior surface of the inferior turbinate; TCR: total chloride response.

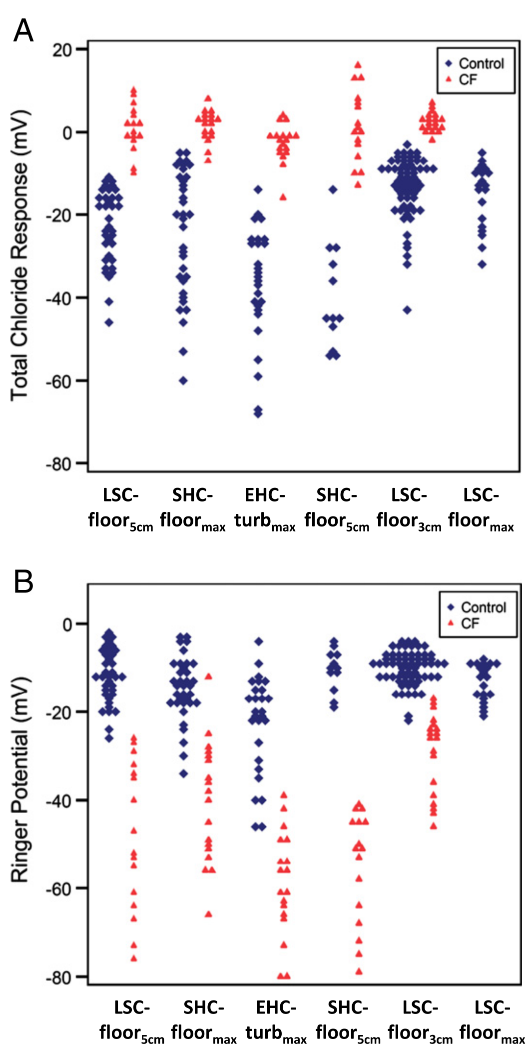

Fig. 4. TCR (A) and Ringer's PD (B) measured in individual nostrils using the different methods in the control subjects (blue diamonds) and in patients with cystic fibrosis (red triangles). CF: cystic fibrosis; EHC: endhole catheter; LSC: larger surface catheter; SHC: sidehole catheter; turb: inferior surface of the inferior turbinate; TCR: total chloride response.

3.4. Mean potentials

The mean values for the Ringer's PD and the TCR for each technique are given in Table 1. The individual values for the control subjects and the patients with CF are shown in Fig. 4.

In the controls, the mean TCR was significantly higher with the EHC-turb$_{max}$ method (-36.6 ± 13.8 mV) or when measured with the side-hole catheter on the nasal floor at 5 cm deep (-40.1 ± 12.5 mV) than on the nasal floor with the larger surface catheter (-13.3 ± 7.3 mV at 3 cm deep, $p = 0.002$, and -22.9 ± 9.0 mV at 5 cm deep, $p < 0.001$). Using the larger surface catheter, the measurements at a fixed depth of 5 cm (new method) resulted in a higher TCR than at 3 cm (-22.9 ± 9.0 mV vs. -13.3 ± 7.3 mV, $p = 0.003$). In the patients with CF, there was no significant difference in the TCR between the different measurement techniques.

3.5. Power calculations

The sample sizes were estimated assuming a 1-sample t-test for the difference between the baseline and follow-up measurements. Different levels of improvement of the parameters were hypothesized based on the range of values observed in the ivacaftor trial [15]. The results are reported in Table 2, showing lower sample sizes for most parameters with the new LSC-floor$_{5\ cm}$ method compared with the SHC-floor$_{max}$ and EHC-turb$_{max}$ methods, but similar sample sizes when the TCR was measured with the sidehole catheter at 5 cm depth on the nasal floor or with the new method.

4. Discussion

Compared with previous methods, the repeatability of the NPD measurement was much improved by using a larger surface catheter placed at a fixed location. This improvement in the repeatability increased the power of the NPD measurement as a biomarker to detect changes in CFTR activity.

Different hypotheses could explain the improvement in the repeatability. First, using a fixed location for the measurement

Table 2
Sample size calculations.

	Improvement (towards values in controls)	LSC-floor$_{5\ cm}$	SHC-floor$_{max}$	EHC-turb $_{max}$	SHC-floor$_{5\ cm}$	LSC-floor$_{3\ cm}$
Total chloride response	10%	51	49	49	33	46
	20%	13	21	17	11	14
	30%	6	15	10	5	8
Ringer's PD	10%	63	273	152	136	84
	20%	14	59	33	30	19
	30%	6	23	13	12	7
Delta NPD	10%	35	63	43	39	46
	20%	8	19	11	10	12
	30%	4	10	6	5	6

CF: cystic fibrosis; EHC: endhole catheter; LSC: larger surface catheter; neg PD: negative potential difference; SHC: sidehole catheter; turb: inferior surface of the inferior turbinate.

instead of a variable location could reduce the variability of the measurement. Ion transport is not uniform throughout the nasal cavity. According to early work from Knowles et al. [18], measurements were initially taken from the inferior surface of the lower turbinate because the basal PD was the most negative and the mucosal histology showed the highest proportion of ciliated cells. However, the insertion of the catheter under the lower turbinate was considered technically difficult by others, leading to alternative protocols measuring the nasal floor [19]. Similar results were obtained when both methods were compared [11]. Measuring at different locations instead of a fixed area on different testing occasions may logically lead to more variable results. In the present study, the repeatability was indeed slightly worse when the measurement was taken from the location of the most negative basal value than from a fixed location, both with a side-hole catheter and with the larger surface catheter. In addition, the usefulness of searching for an 'optimal' location is questionable because the TCR in the controls was larger when using a fixed location rather than the location with the most negative basal potential.

Second, measuring a larger surface area results in an averaged value that is more likely to be repeatable in a subsequent measurement.

Third, the properties of the catheters could have played an important role. The larger surface catheter has a larger diameter than side-hole or Marquat catheters, preventing its displacement during measurement. With smaller catheters, the contact with the nasal mucosa could be less consistent, and minor differences in the orientation of the opening could impact the measured values.

Before the nasal potential difference can be used as a biomarker, this technique requires rigorous standardization. However, even with standardization, small differences in practice can have a major impact on the results obtained [20]. NPD values may vary considerably between operators according to factors that are difficult to standardize such as the exact location of the catheter, the care taken not to injure the mucosa during catheter insertion, or stabilization of the catheter during measurement. A 'real life' evaluation of the feasibility of NPD measurement and its accuracy and reliability in the hands of less-experienced operators should prove that the proposed technique is appropriate for generalization. The use of a larger surface catheter placed at a fixed location appears to be simpler than using a small catheter, which is prone to displacement, under the turbinate, a location that is more difficult to reach. In addition, the use of a larger, firmly secured catheter allows simultaneous measurements in both nostrils, halving the measurement time.

The amplitude of the TCR was higher when measured with a small catheter than with the larger surface catheter. This result is understandable because the latter averages the potentials over a larger surface area, precluding the recording of a 'peak' value, which would be the case with a small catheter. It is important to note that the high values we measured using the EHC$_{turb}$ method have not universally been found by others. A TCR of only −25 mV was observed in control subjects in a multicenter trial using a similar protocol [21], close to the value we found with the larger surface catheter. The high values we obtained could be due to subject characteristics (mainly young subjects in our cohort) or residual methodological differences.

The repeatability of each method will be further increased by averaging the values measured in each nostril [19]. Obtaining more repeatable results from each side could allow to evaluate other approaches than averaging both sides, such as using the most polarized nostril [15].

The strengths of the present study were the large number of measurements. A single experienced operator performed all of the measurements, thereby avoiding the inter-operator variability inherent to comparisons between different operators using different techniques. The repeatability obtained with the SHC-floor$_{max}$ and EHC-turb$_{max}$ methods was similar to previously published values [15,22]. The major weakness was that different subjects were included in the evaluation of the different techniques. From previous studies, we knew that repetition of the nasal potential measurements was likely to lead to a high dropout rate, especially in the control subjects. Another possible hurdle in the implementation of the new method is the use of a 'home made' catheter, which could rise regulatory issues, as has been the case for the 'Marquat' catheter in the Unites States. Before further evaluation of a new method is started, designing a catheter that will obtain approval by regulatory issues is an important step. An alternative could be to assess the repeatability using the actual SOPs and catheters, but taking the measurement from a fixed location, and not the location with the most negative basal potential.

We concluded that measuring the NPD both with a larger surface catheter or at a fixed location, improved the repeatability of the measurement, thereby reducing the sample size needed in clinical trials. Future research should include multi-center evaluations of these methodological adaptations to demonstrate the superior power to detect therapeutic effects in patients with CF.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcf.2014.08.006.

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